EFFECTS OF ARBUSCULAR MYCORRHIZA, BIOCHAR, AND VERMICOMPOST ON MAIZE (ZEA MAYS) IN SALINE SOIL

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Abstract

Soil salinity is a great constraint for crop production in the coastal ecosystem of Bangladesh. Both cropping intensity and crops yield are very low in the coastal areas. The present study was undertaken to identify the effect of native Arbuscular Mycorrhizal Fungi (AMF), biochar, and vermicompost on biomass, fungal colonization, and yield characters of maize in moderately saline soil (EC value 8 dS m^{-1}). A pot experiment was performed in the net-house of Soil Science Division, Bangladesh Agricultural Research Institute (BARI), Gazipur in 2022 and 2023. The test variety was BARI Hybrid Maize-9. The experiment was designed in a completely randomized design with 10 treatments and four replications. The treatments were T_1: Control, T_2: Arbuscular mycorrhiza (AM), T_3: Biochar @ 10 t ha^{-1}, T_4: Vermicompost @ 3 t ha^{-1},T_5: AM + Biochar @ 5 t ha^{-1}, T_6: AM + Biochar @ 10 t ha^{-1}, T_7: AM + Vermicompost @ 3 t ha^{-1}, T_8: AM + Vermicompost @ 6 t ha^{-1}, T_9: Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1} and T_{10}: AM + Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1}. The result showed that AM + Vermicompost @ 3 t ha^{-1} produced the highest biomass, colonization, and yield characteristics of maize, while the control recorded the lowest. Treatment T_7 produced the highest kernel yield of maize which was 92% and 62% higher over the control in 2022 and 2023, respectively. This result was significantly higher in the T_7 treatment compared to all other treatments. Therefore, the combined use of AM fungi and vermicompost could sustain soil health and ensure better crop productivity in a saline environment of Bangladesh.

Keywords: Biomass, Fungal colonization, Electrical conductivity, Maize yield.

1. Introduction

Soil salinity, a global land degradation problem, significantly reduces agricultural productivity. With the increasing sea levels due to global warming, the area covered by salt-affected soils is on the rise trend. In Bangladesh, for instance, out of 2.86 million hectares, around 1.06 million ha of land in the southern coastal region is salt-affected (SRDI, 2010).

To resolve this rising concern, we underscore using biological soil amendments such as arbuscular mycorrhizal fungi, biochar, and vermicompost at different rates. Plants inoculated

with mycorrhiza have been reported to increase plant growth and yield under salinity or NaCl stress conditions, such as *Ocimum basilicum* (Ashoori *et al.,* 2015) and *Lens culinaris* (Rahman *et al.,* 2017). Biochar is a porous, fine-grained substance similar to charcoal and decomposes much more slowly than other organic matter in the soil. Although biochar has little plant nutrient content, its high surface area and porous structure increase the soil surface area, provide a habitat for beneficial soil microorganisms, aid in water retention, and reduce leaching out of nutrients. All of these functions increase the availability of nutrients to plants (Schahczenski, 2010). Vermicompost is a hummus-like substance formed when organic matter is broken down by the joint action of earthworms and microorganisms (Lazcano *et al.,* 2008).

Maize in Bangladesh has appeared as an important cereal crop. A good number of experiments have been conducted so far using mycorrhizal fungi, biochar, and vermicompost. For instance, the findings of Dawar *et al.* (2002) suggested that biochar and vermicompost can increase maize growth and yield characteristics as a sole amendment. However, their co or combined application in the presence of recommended NPK is an excellent strategy for enhancing maize growth in poor organic matter soils. Tafara *et al.* (2017) found that a remarkable reduction in plant performance was noted when biochar was applied at 100%. He recommended using biochar as a bio-fertilizer at 50/50% biochar: vermicompost in maize and cabbage production. Song *et al.* (2022) experimented using biochar and vermicompost. He depicted that using vermicompost showed better results than using biochar alone or the biochar-compost combination. Besides, Karami *et al.* (2018) study explored the fact that the combined application of mycorrhizal fungi and vermicompost increased the yield of maize. So, we hypothesized that using AM fungi, biochar, and vermicompost can improve maize yield in saline conditions which yet to be explored. Hence, the present investigation was carried out to evaluate the effect of indigenous Arbuscular Mycorrhizal Fungi (AMF), biochar, and vermicompost on biomass, nodulation, colonization, and yield characters of maize in saline soil.

2. Materials and Methods

2.1 Experimental location and season

The experiment was conducted during January to June 2022 and 2023 in the net-house of Soil Science Division, BARI, Joydebpur, Gazipur (23^0 59'38'' N latitude, 90^0 24'89'' E longitude and 8.4 m elevation).

2.2 Seed collection and soil preparation

Seeds of maize (BARI Hybrid Maize-9) were collected from Bangladesh Wheat and Maize Research Institute (BWMRI), Regional Office, Gazipur. The silted (sandy clay loam) soils were collected from the bank of Turag River at Kodda, Gazipur and soil was mixed with cowdung at a 5:1 ratio. Each pot (28 cm in diameter and 23 cm in height) was filled with eight kg soil. Table 1 represents the physical and chemical properties of the soil and that of cowdung.

Table 1. Initial fertility status of soil and cowdung used in the pot experiment

Samples	Texture	pH	OM (%)	Ca	Mg	K	Total N (%)	P	S	B	Cu	Fe	Mn	Zn
				meq 100 g^{-1}				μg g^{-1}						
Soil	Sandy clay loam	7.1	0.51	7.2	2.5	0.11	0.026	9.9	21.1	0.22	1.8	15	1.1	0.38
Critical level	-	-	-	2.0	0.5	0.12	-	10	10	0.20	0.2	4.0	1.0	0.60
Cowdung	-	6.7	14.1	1.55	0.82	0.88	0.84	1.26	0.62	0.02	0.01	0.25	0.11	0.02

2.3 Soil analysis

Soil pH was measured by a combined glass calomel electrode (Jackson, 1962). Organic carbon was determined by wet oxidation method (Walkley and Black, 1934). Total N was measured by the modified Kjeldahl method (Jackson, 1962). Calcium, K, and Mg were determined by the NH4OAc extraction method (Black, 1965). Copper, Fe, Mn, and Zn were determined by DTPA extraction followed by Atomic Absorption Spectrophotometry (AAS). Boron was determined by $CaCl_2$ extraction method (Page *et al.,* 1982). Phosphorus was determined by Modified Olsen method (Olsen *et al.,* 1954). Soil S was determined by $CaH_4(PO_4)_2.H_2O$ extraction followed by turbidimetric turbidity method with $BaCl_2$ (Chesnin and Yien, 1950).

2.4 Preparation of salinity solution and production of mycorrhizal biofertilizer

Required salinity concentrations were prepared according to New South Wales (NSW), Australia, and applied thrice during experimentation. Firstly, before transplanting the maize seedlings, one month after transplanting, and finally, before the flowering stage of the maize plants. The soil-based AM fungal inoculum containing 150 g of rhizosphere soil (approximately 252 ± 20 spores/100 g soil) and infected sorghum root fragments with a minimum colonization level was inoculated to each mycorrhizal pot. The mycorrhizal inoculum was placed in each pot at 3-5 cm depth. It was covered with a thin soil layer of 1 cm immediately before the three maize seedlings transplantation to facilitate fungal colonization of plant roots. Flow diagram 1 illustrates the production of mycorrhizal biofertilizer.

2.5 Identification of AM fungal spores

AM fungal spore, single spore or sporocarps were easily picked up from the Petridish by extrude pipette and mounted on a glass slide with a drop of polyvinyl lactophenol (PVL) and a cover slip was placed. Subsequently, recovered spores were distinguished or identified with the help of a Manual and different taxonomic keys proposed by different workers (Schwarzott *et al.,* 2001). For identification of spore or sporocarps we considered spore morphology, size, shape and peridium of spore, sporocarps colour, wall ornamentation, subtending hyphae and mode of attachment.

2.5 Fertilizer application

Chemical fertilizers were applied as a soil test basis according to the method described in the fertilizer recommendation guide (BARC, 2018). All fertilizer except urea was used as basal during final land preparation, and urea was applied as a top dressing in two equal splits 25 and 45 days after sowing. Mycorrhiza, Biochar, and Vermicompost were collected from the Soil Science Division, BARI, Gazipur, and applied as per the requirement described in the treatment. The chemical composition of biochar and nutrient status of vermicompost used in the investigational pot are presented in Table 2.

Table 2. Chemical composition of biochar and vermicompost used in the experiment

Organic material	OC%	N%	Ca%	Mg%	K%	P%	S%
Sawdust based biochar	91.5	1.08	3.85	1.59	1.16	0.55	0.21
Vermicompost	15.1	2.04	2.12	1.19	1.06	1.16	0.33

2.6 Design of experiment and treatments

The experiment was designed in CRD with ten treatments and four replications. The ten treatments were T_1 : Control (not absolute control), T_2 : Arbuscular mycorrhiza (AM), T_3 : Biochar @10 t ha^{-1}, T_4 : Vermicompost@ 3 t ha^{-1},T_5 : AM + Biochar @ 5 t ha^{-1}, T_6 : AM + Biochar @ 10 t ha^{-1}, T_7 : AM + Vermicompost @ 3 t ha^{-1}, T_8 : AM + Vermicompost @ 6 t ha^{-1}, T_9 : Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1} and T_{10} : AM + Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1}. Maize seeds were initially sown in the seedbed using mycorrhiza (@ 1 kg m^2) and non-mycorrhiza. After 14 days, seedlings raised in the mycorrhizal bed were transferred to the AM treatment pots, and seedlings raised in the non-mycorrhizal bed were transferred to without the AM treatment pots. Finally, the treatments were sustained with 03 well-established vigorous seedlings pot^{-1}.

Crop (maize) was harvested at maturity. Subsequently, growth parameters, biomass, colonization, yield and yield contributing characters, and spore population were recorded.

2.7 Assessment of spore population and root colonization (%)

The Olympus SZX10 microscope and Olympus BX41 fluorescence microscope were used for calculating spore population and root cononization (%). The spore population was assessed following the Wet Sieving and Decanting Method (Gerdemann and Nicolson, 1963). All the AM spores were isolated from the extract with the help of fine forceps and put into a watch glass with a small quantity of water. The extract, with AM spores, was observed under a stereomicroscope, and the number of spores was counted. Spore numbers from the three replicates per sample were averaged, and the result was expressed as a number per 100 g of dry soil basis. The root slide technique estimated the percentage of AM colonization (Read *et*

al., 1976). A root segment was considered positively infected if it showed mycelium, vesicles, and arbuscules, or any other combination of these structural characteristics of AM colonization. The presence or absence of colonization in the root pieces was recorded, and the percent colonization was calculated by dividing the number of AM-positive segments by the total number of segments scored and multiplying this value by 100.

2.8 Statistical analysis

Experiment data were statistically analyzed using Analysis of Variance (ANOVA) following the Statistix 10 package. Correlation was done by the pearson method utilizing open source software R version 4.2.2 and RStudio.

3. Results

3.1 Effects on growth, biomass, colonization and spore population of maize

The effects of Arbuscular Mycorrhizal Fungi (AMF), biochar, and vermicompost on growth parameters, biomass, colonization, and spore population of maize are presented in Table 4 and Figs. 1-4. Significant differences were found in all the parameters except leaf number plant^{-1} in both years and plant height and root fresh weight in 2023. In 2022, the highest plant height (217 cm), leaf number (12.7 plant^{-1}), root fresh weight (117.5 g plant^{-1}), shoot fresh weight (161.8 g plant^{-1}), root oven dry weight (25.8 g plant^{-1}), shoot oven dry weight (79.7 g plant^{-1}), root colonization (53.3 %) and spore population (67.5, 100 g^{-1} soil) were observed in AM + Vermicompost @ 3 t ha^{-1} treatment. The lowest plant height (201 cm), leaf number (12.1 plant^{-1}), root fresh weight (85.3 g plant^{-1}), shoot fresh weight (130 g plant^{-1}), root oven dry weight (17.8 g plant^{-1}), shoot oven dry weight (57.7 g plant^{-1}), root colonization (3.34 %) and spore population (30.5, 100 g^{-1} soil) were observed in the control treatment.

In 2023, the highest plant height (213 cm), leaf number (10.7 plant^{-1}), root fresh weight (30.4 g plant^{-1}), shoot fresh weight (133.8 g plant^{-1}), root oven dry weight (17.8 g plant^{-1}), shoot oven dry weight (61.5 g plant^{-1}), root colonization (50.0 %) and spore population (76.0, 100 g^{-1} soil) were observed in AM + Vermicompost @ 3 t ha^{-1} treatment. The lowest plant height (198 cm), leaf number (9.58 plant^{-1}), root fresh weight (24.6 g plant^{-1}), shoot fresh weight (106 g plant^{-1}), root oven dry weight (13.8 g plant^{-1}), shoot oven dry weight (46.1 g plant^{-1}), root colonization (12.5 %) and spore population (43.5, 100 g^{-1} soil) were observed in control treatment.

Table 4. Effect of Arbuscular Mycorrhizal Fungi (AMF), biochar and vermicompost on growth parameters, fresh root and shoot biomass of maize

Treatments	Plant height (cm)		Leaf number plant^{-1}		Root fresh weight (g plant^{-1})		Shoot fresh weight (g plant^{-1})	
	2022	2023	2022	2023	2022	2023	2022	2023
T_1	201c	198	12.1	9.6	85.3b	24.6	130b	106c
T_2	201c	207	12.7	10.3	88.5b	29.8	137b	124abc
T_3	215ab	212	12.7	10.4	89.3b	30.3	143b	134a
T_4	216a	212	12.7	10.5	117.1a	30.0	161a	130ab
T_5	215a	211	12.7	10.3	84.6b	25.0	132b	106c
T_6	215ab	211	12.2	10.6	85.8b	30.1	131b	122abc
T_7	217a	213	12.7	10.7	117.5a	30.4	162a	134a
T_8	214ab	213	12.3	10.3	114.4a	29.3	134b	127abc
T_9	205abc	209	12.6	10.0	90.6b	27.2	161a	112bc
T_{10}	202bc	202	12.3	10.0	85.8b	25.9	137b	109c
SE (±)	4.42	6.67	0.20	0.39	5.93	2.34	4.81	7.20
F test	*	ns	ns	ns	**	ns	**	*
CV (%)	4.20	6.39	3.13	7.54	12.36	16.60	6.75	12.00

T_1 : Control, T_2 : Arbuscular mycorrhiza (AM), T_3 : Biochar @ 10 t ha^{-1}, T_4 : Vermicompost @ 3 t ha^{-1}, T_5 : AM + Biochar @ 5 t ha^{-1}, T_6 : AM + Biochar @ 10 t ha^{-1}, T_7 : AM + Vermicompost @ 3 t ha^{-1}, T_8 : AM + Vermicompost @ 6 t ha^{-1}, T_9 : Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1} and T_{10} : AM + Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1}. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. **Significant P≤0.01, *Significant P≤0.05, ns non significant.

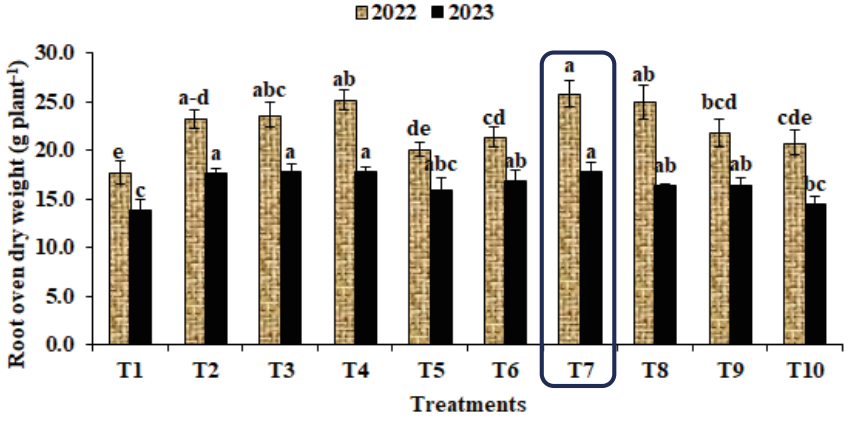

Fig. 1 Effects of Arbuscular Mycorrhizal Fungi (AMF), biochar and vermicompost on root oven dry weight of maize. T_1 : Control, T_2 : Arbuscular mycorrhiza (AM), T_3 : Biochar @10 t ha^{-1}, T_4 : Vermicompost @ 3 t ha^{-1}, T_5 : AM + Biochar @ 5 t ha^{-1}, T_6 : AM + Biochar

@ 10 t ha^{-1}, T_7 : AM + Vermicompost @ 3 t ha^{-1}, T_8 : AM + Vermicompost @ 6 t ha^{-1}, T_9 : Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1} and T_{10} : AM + Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1}.

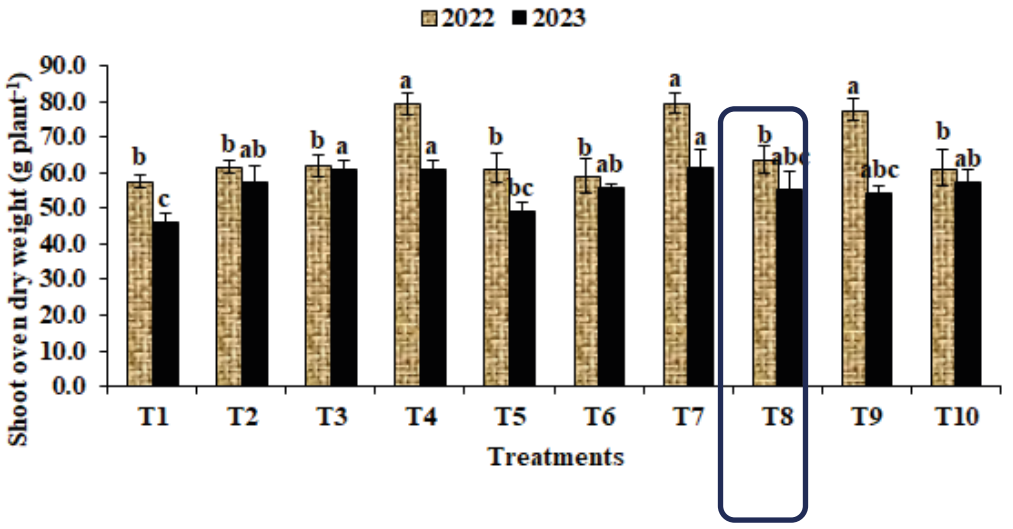

Fig. 2 Effects of Arbuscular Mycorrhizal Fungi (AMF), biochar and vermicompost on shoot oven dry weight of maize.

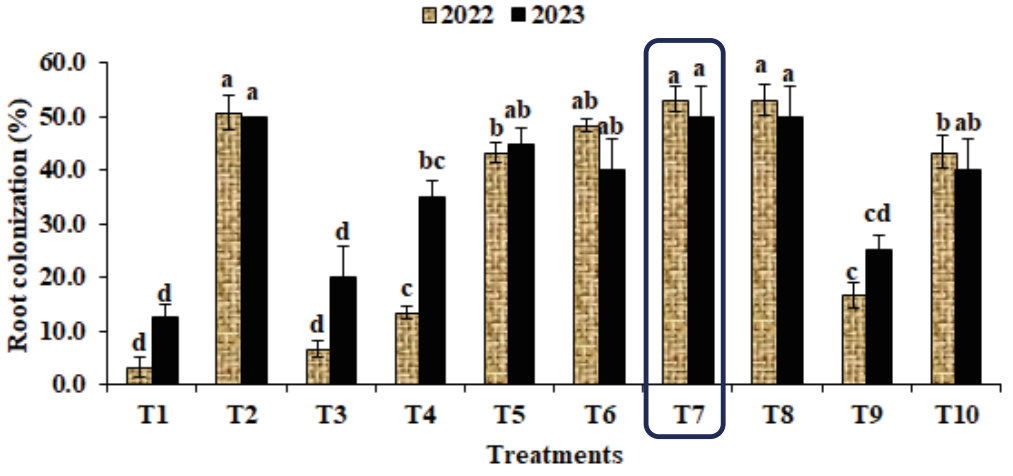

Fig. 3 Effects of Arbuscular Mycorrhizal Fungi (AMF), biochar and vermicompost on root colonization (%) of maize.

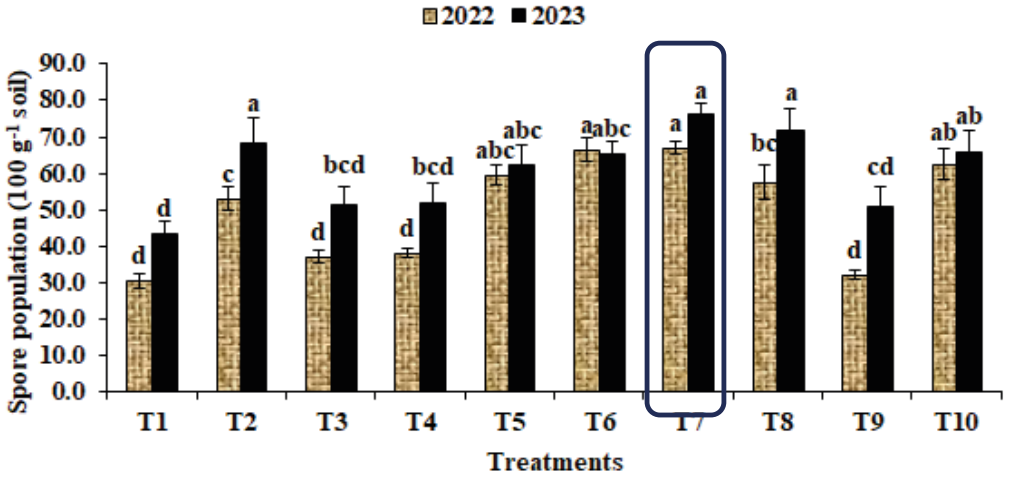

Fig. 4 Effect of Arbuscular Mycorrhizal Fungi (AMF), biochar and vermicompost on spore population of maize.

3.2 Effects on yield and yield contributing characters of maize

Results on the effect of Arbuscular Mycorrhizal Fungi (AMF), biochar, and vermicompost on yield and yield-contributing characteristics of maize are presented in Table 5 and Fig. 5. Significant differences were found in all the parameters except ear length and number of rows ear^{-1} in both years and 50 kernel weight in 2023. In 2022, the highest ear length (10.2 cm), number of rows (18.1 ear^{-1}), number of kernel (499 pot^{-1}), 50 kernel weight (12.0 g), ear weight (162 g pot^{-1}) and kernel yield (101 g pot^{-1}) were observed in AM + Vermicompost @ 3 t ha^{-1} treatment. The lowest ear length (9.52 cm), number of rows (14.1 ear^{-1}), number of kernels (220 pot^{-1}), 50 kernel weight (10.5 g), ear weight (96.3 g pot^{-1}), and kernel yield (52.8 g pot^{-1}) were observed in the control treatment.

In 2023, the highest ear length (7.98 cm), number of rows (17.5 ear^{-1}), number of kernel (290 pot^{-1}), 50 kernel weight (11.1 g), ear weight (110 g pot^{-1}) and kernel yield (60.0 g pot^{-1}) were observed in AM + Vermicompost @ 3 t ha^{-1} treatment. The lowest ear length (6.75 cm), number of rows (14.0 ear^{-1}), number of kernel (176 pot^{-1}), 50 kernel weight (9.13 g), ear weight (74.5 g pot^{-1}) and kernel yield (37.0 g pot^{-1}) were observed in control treatment. It was noticed that AM + Vermicompost @ 3 t ha^{-1} treatment (T_7) produced the highest kernel yield (101 g pot^{-1}, 91.9% higher over control in 2022 and 60.0 g pot^{-1}, 62.2% higher over control in 2023) of maize which was significantly different from the rest of the treatments.

Table 5. Effects of Arbuscular Mycorrhizal Fungi (AMF), biochar and vermicompost on yield and yield contributing characters of maize

Treatments	Ear length (cm)		No. of rows ear^{-1}		No. of kernel pot^{-1}		50 kernel weight (g)		Kernel yield increase over control (%)	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
T_1	9.52	6.75	14.1	14.0	220d	176c	10.5c	9.13	-	-
T_2	9.71	7.69	16.0	15.7	254d	237b	11.5abc	9.14	6.64	33.8
T_3	9.85	7.61	16.7	16.4	346c	247b	11.7ab	9.74	43.6	46.0
T_4	9.88	7.87	17.3	16.8	363bc	267ab	11.9a	9.98	47.9	59.5
T_5	9.71	6.77	17.4	14.2	355bc	258ab	10.8bc	9.43	47.4	36.5
T_6	9.67	7.71	16.7	14.6	367bc	255ab	10.8bc	9.72	63.0	43.2
T_7	10.17	7.98	18.1	17.5	499a	290a	12.0a	11.1	91.9	62.2
T_8	10.08	7.78	17.8	17.5	396b	241b	11.0abc	9.99	64.0	56.1
T_9	9.75	6.83	16.3	14.2	343c	260ab	10.5c	9.35	21.8	43.2
T_{10}	9.83	6.75	15.1	14.2	257d	245b	10.5c	9.68	4.74	29.1
SE (±)	0.38	0.53	1.08	1.08	16.78	14.10	0.40	0.50	-	-
F test	ns	ns	ns	ns	**	**	*	ns	-	-
CV (%)	7.69	14.33	13.01	13.95	9.88	11.4	7.13	10.4	-	-

-T_1: Control, T_2: Arbuscular mycorrhiza (AM), T_3: Biochar @10 t ha^{-1}, T_4: Vermicompost@ 3 t ha^{-1}, T_5: AM + Biochar @ 5 t ha^{-1}, T_6: AM + Biochar @ 10 t ha^{-1}, T_7: AM + Vermicompost @ 3 t ha^{-1}, T_8: AM + Vermicompost @ 6 t ha^{-1}, T_9: Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1} and T_{10}: AM + Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1}. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. **Significant P≤0.01, *Significant P≤0.05, ns non significant.

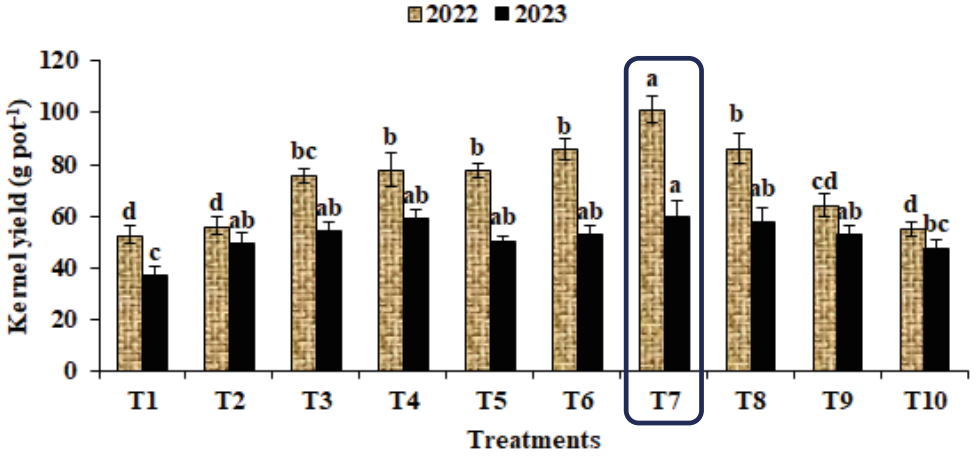

Fig. 5 Effects of Arbuscular Mycorrhizal Fungi (AMF), biochar and vermicompost on kernel yield of maize.

T_1 : Control, T_2 : Arbuscular mycorrhiza (AM), T_3 : Biochar @10 t ha^{-1}, T_4 : Vermicompost @ 3 t ha^{-1}, T_5 : AM + Biochar @ 5 t ha^{-1}, T_6 : AM + Biochar @ 10 t ha^{-1}, T_7 : AM + Vermicompost @ 3 t ha^{-1}, T_8 : AM + Vermicompost @ 6 t ha^{-1}, T_9 : Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1} and T_{10} : AM + Biochar @ 5 t ha^{-1} + Vermicompost @ 3 t ha^{-1}.

4. Discussion

AM + Vermicompost @ 3 t ha^{-1} treatment (T_7) produced the highest yield because the same treatment produced the higher plant biomass, and the higher biomass was for consequently higher sporulation and colonization that bolster rhizospheric microbiome activity and enhanced nutrient acquisition by maize plant. These results are supported by the findings of Warnock *et al.* (2010), Copetta *et al.* (2011) and Cavender *et al.* (2003).

As can be seen, mycorrhizal inoculation results in better yield than non-mycorrhizal treatments under saline conditions. The findings of Rahman *et al.* (2019) depicted that arbuscular mycorrhizal colonization increases yield by reducing the adverse effects of salinity up to 6 dS m^{-1}, and this increased yield and yield traits associated with increased dry biomass, nodulation, colonization, nutrient concentration, and uptake of the plant under AM inoculation. This increase in yield could be due to vermicompost, which is used as an amendment with high levels of nutrient content. Jain *et al.* (2012) reported that the application of vermicompost increased the soil's N, P, and K content.

Soil amendments, along with mycorrhiza and vermicompost, enhanced maize's ear weight and kernel yield. These results support an experiment by Laufer and Tomlinson (2013), where biochar's application improved maize yield over the control plot by 2.2 tons per hectare. Shishehbo *et al.* (2013) also showed that biological yield was higher when vermicompost was applied along with azotobacter and arbuscular mycorrhizal fungi; however, according to Copett et al. (2011), the best dry weight yield occurred at compost rates of 75% and AM fungi application.

5. Conclusions

The result showed that AM + Vermicompost @ 3 t ha^{-1} treatment produced the highest growth parameters, biomass, colonization, and yield characteristics of maize in 8 dS m^{-1} saline soil, and the control treatment produced the lowest growth parameters, biomass, colonization, and yield characters of maize in saline soil. The T_7 treatment produced the highest kernel yield of maize showing 101 g pot^{-1} which 91.9% higher over control in 2022 and 60.0 g pot^{-1} exhibiting which 62.2% higher over control in 2023. This result was significantly higher over that of the other treatments. Therefore, the combined use of AM fungi and vermicompost could sustain soil health and ensure better crop productivity in a saline environment of Bangladesh. We justified our research objectives and achieved tremendous

results even though the pure culture strain of AM fungal inoculum production constrained us. Benefits include saving capital costs and helping to achieve sustainable agriculture and food security in Bangladesh. It is strongly suggested that environmental factors be addressed for rigorous further research.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

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